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Presented in Partial Fulfillment of the
Degree of Doctor of Medicine

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March 1977

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Acknowledgments

First, I would like to express my sincere gratitude towards my advisor, Dr. Norman J. Siegel. His advice, encouragement, and good humor were greatly appreciated.

Also I would like to thank Dr. Mark Osias, Ms. Sonia Gunstream, Ms. Barbara Goldberg, and Ms. Theresa An for their invaluable technical assistance, and Ms. Robin Jackoway for her expert typing skills.

Introduction

Over the centuries, the concept of regeneration of lost organs has fascinated man. Although careful observation of such regeneration was done (such as the ability of a starfish to grow a lost limb), that which occurred in higher organisms was not scientifically studied until the twentieth century. Obviously, though, research on compensatory renal alterations was hampered by lack of understanding of renal physiology, and the concepts of glomerular filtration rate (GFR) and renal blood flow (RBF). Over the past 25 years, the subcellular world of electron microscopy has provided new precepts for researchers in compensatory renal alterations. This thesis will attempt to trace the course of this research from a time when the only studiable parameter was renal weight. Then some new experimental data comparing compensatory renal function and growth between unilaterally obstructed and nephrectomized animals will be presented and discussed in relation to the current knowledge and theories on the subject.

For simplicity, this thesis is divided into two sections. The first, a review of the literature, discusses the alterations in mass, functional and histological changes, and biochemical alterations seen following loss of functional renal tissue. Four theories explaining these changes will be presented and

discussed. The second section is devoted to the materials and methods involved in performing a new experiment, the results, and a discussion of the new studies.

REVIEW OF THE LITERATURE

I. STUDIES

Alterations in Renal Mass

Although the phenomenon of compensatory renal function had been recognized since before the turn of the century, it was not until Hinman's classic work in 1922 that the first really objective study was performed. He noted that hypertrophic increases in renal volume secondary to contralateral ureteral obstruction in rats were complete after 20-30 days (Hinman, 1922). At about the same time, others showed that following unilateral nephrectomy, the remaining kidney weighed 58% of the weights of both kidneys at 15-33 days, and 66% of both kidneys after 106 days (Oliver, 1924; Addis et. al., 1924). Normally, one kidney should weigh about 50% of the total renal mass.

Moise and Smith concluded that the greatest increase in renal mass (20% over control) occurred during the first 3 weeks post-unilateral nephrectomy, continued slowly up to 4 months, and stopped after that, and that high casein diets increased the amount of hypertrophy (Moise and Smith, 1926; Smith and Moise, 1927); but Jackson and Levine suggested that early hypertrophy was biphasic, with an actual transient decrease in renal weight after 3 days, and a permanent increase

beginning after 7 days. Although they concluded that the initial weight increase during the first few days post-unilateral nephrectomy was due to the transient pseudohypertrophy of congestion (Jackson and Levine, 1929), their work has only recently been corroborated (Kurnick, 1968).

When old and young rats were compared, both groups were seen to complete their compensatory increase in weight by about 40 days after nephrectomy, but the amount of hypertrophy was greater in younger than in older rats (Jackson and Shiels, 1927; MacKay et. al., 1932; MacKay et. al., 1938; Addis and Lew, 1940; Galla, 1974). Also recently, it has been shown that at all times during the course of compensatory renal growth, the dry kidney weight is always 24% of the wet kidney weight, thus making either parameter just as valid as the other for study (Malt and LeMaitre, 1968).

In humans, IVP's performed pre-operatively on transplant donors and again post-operatively, showed an overall increase in kidney length of 8%, renal area of 16.8%, with no difference seen between donors under 40 and those over 40 (Boner et. al., 1972; Orecklin et. al., 1973; Donadio, 1967).

Recently, Kaufman and Hayslett have demonstrated that the amount of compensatory renal growth is proportional to the amount of kidney removed, by showing greater proportional increases in renal mass at one month post-removal of 1 1/2 kidneys than following removal of only one kidney (Hayslett,

1972; Kaufman, 1974, 1975). On the other hand, studies involved with just the opposite (i.e. adding renal mass to healthy animals), have yielded fruitful data. Silber transplanted extra kidneys from litter-mates into healthy baby rats, 4 to 5 weeks of age. When the donor kidneys were from rats of the same age, 18 weeks after transplantation the 3 kidneys were equal in weight and each kidney weighed the same as kidneys from control rats. When the donor kidneys were from adult rats, the baby rats' original kidneys still grew normally, and 9 weeks post-operatively were equal in weight to those in two-kidney controls. However when kidneys from baby rats were transplanted into adult rats, 16 weeks post-surgery the transplanted kidneys had decreased growth when compared with that seen in the kidneys of the donor rats' healthy litter-mates. Also, when hypertrophied kidneys from unilaterally nephrectomized adult rats were transplanted into other unilaterally nephrectomized adult rats (thus creating animals with two hypertrophied kidneys), 6 weeks after transplant both kidneys had shrunk to normal size. From all of this, Silber postulated that there were two types of renal growth: obligatory and compensatory. Obligatory renal growth occurred slowly along with the animals' growth and was not reversible, thus explaining why the kidneys in 3-kidney baby rats did not shrink. Compensatory renal growth occurred secondary to reductions in the nephron population and was reversible after restoration of the deficit (Silber, 1974; Silber and Malvin, 1974).

Although the many studies cited above clearly demonstrated compensatory renal growth following either unilateral nephrectomy or ureteral obstruction, a relatively small amount of work has been done comparing the amounts of growth between these two groups. Recently, Veeder's work showed that at 21 days post-unilateral obstruction the weight of the intact kidney had increased 65% while the weight of the remaining kidney in unilaterally nephrectomized rats had increased 80% (Veeder, 1975).

Finally, although Mason and Ewald demonstrated that contralateral kidney growth post-ureteral ligation was much slower than growth post-nephrectomy (Mason and Ewald, 1965), more recent work has indicated that despite greater absolute weight increases following nephrectomy, the rates of growth post-ligation vs. post-nephrectomy were actually the same (Dicker and Shirley, 1972).

Thus, following either unilateral nephrectomy or obstruction, the greatest compensatory increase in renal mass appears to occur within the first 3 weeks post-surgery to give a renal weight about 60% of the pre-op total renal weight. Little growth appears to occur after this time. Also some data suggest that there is a greater absolute increase in weight seen following unilateral nephrectomy when compared with that seen after obstruction of a ureter.

Functional Changes

In the 1920's and '30's Addis and others noted that following unilateral nephrectomy in animals, urea excretion was 69% of normal at 15-33 days and 98% of normal after 106 days, but that urea clearance was the only parameter that correlated with changes in renal weight so that it was 63% of normal at 15-33 days and 79% after 106 days (Addis, 1924; Rhoads, 1934). Van Slyke noted that one week post-nephrectomy, the urea clearance per gram of kidney was 143% of pre-nephrectomy values in dogs (Van Slyke, 1934). It was also demonstrated that an 80% reduction in total renal mass produced a BUN of 50-80 mg% and dilute urine, while a greater than 80% reduction led to a BUN of 120-150 mg% and uremia in rabbits (Drury, 1932).

However, blood flow studies done around the same time have been somewhat less consistent. Although 2 and 3 hours post-nephrectomy the RBF to the remaining kidney did not seem to increase (Herrick, 1932; Fajers, 1957), one week post-op the average perfusion per gram of kidney was noted to be 168% of pre-nephrectomy values and renal oxygen consumption per gram was 181% of controls (Van Slyke, 1934). However, various studies have shown renal blood flow and O_2 consumption reached its maximum either 1 month (Van Slyke, 1934), 2 months (Maluf, 1949) or 4 months (to a value of 200% of pre-op values) after

unilateral nephrectomy (Levy and Blalock, 1938). Coincidentally, blood flow to unilaterally obstructed kidneys was shown to decrease (Herdman, 1950; Idbhorn, 1956; Moody, 1975; Lyrdal, 1975; Harris, 1974; Vaughan, 1970), and increase to 68% of single kidney levels after relief of obstruction (Kerr, 1954).

Studies on GFR and RBF during the first 24 hours post-nephrectomy have been somewhat contradictory. Although several groups of workers have shown that 1 hour post-op, the GFR and T_{Na} was 60-75% of 2-kidney control, the same groups differed as to 18 hour values (60% vs. 85%) (Potter, 1969; Wagenknecht, 1971). Also Wagenknecht, using radioactive tracers, found no change in RBF to the remaining kidney 1 hour post-unilateral nephrectomy, and a slow (10%) increase over 24 hours; while Krohn, using a direct renal artery transducer in dogs got an increase of 27% in the first 5 minutes, with a slow rise to 33% over single kidney pre-op values by 3 hours (Krohn, 1970; Wagenknecht, 1971).

Studies on functional changes after 24 hours also have been inconsistent. Katz and Epstein showed that post-unilateral nephrectomy GFR rose slowly to 65% of 2-kidney values at 3 days, 80% at 7 days and leveled off at 88% after 2 weeks, with T_{Na} closely paralleling the GFR (Katz and Epstein, 1967). Oleson and others found similarly that RBF and T_m PAH also leveled off at 2 weeks to 65% and 57% respectively, with no further changes up to 2 years later (Oleson and Madsen, 1975;

Vaughan, 1970). However, other groups have demonstrated an initial sharply elevated increase in GFR at 1-2 days both post-nephrectomy and post-obstruction to equal 2-kidney pre-op values at 48 hours, followed by a subsequent steady slow decrease until a level of 60-70% of pre-op 2-kidney values was reached in both groups (Dicker and Shirley, 1971, 1972), while Swedish workers concluded that post-unilateral ureteral ligation, the GFR did not change after the first 24 hours! (Lyrdal and Olin, 1975) Rous and Wakim also showed the GFR to decline after an initial increase at 24 hours post-nephrectomy, but they demonstrated a second rise after 4 weeks reaching a plateau at 8 weeks (Rous and Wakim, 1967). Peters also obtained a "biphasic" GFR curve but his second peak leveled off at 3 weeks post-op. He also noted no differences between GFR post-unilateral nephrectomy and post-unilateral ureteral obstruction (Peters, 1963).

However, more recent work seemed to indicate that the rate of increase of GFR and RBF following unilateral obstruction was slower than that following unilateral nephrectomy, but at 1 year post-op they were not statistically different (Oleson and Madsen, 1975 a,b). Indeed Veeder's work showed that at 21 days post-op, the GFR in unilaterally obstructed rats was 62% of 2-kidney values while that in unilaterally nephrectomized rats was 72% of control (Veeder, 1975).

Curiously, when the renal threshold of glucose (proportional to $T_m$gluc/GFR) was studied as an indicator of glomerulo-tubular function, following unilateral nephrectomy in dogs, the same research group got contradictory results. In one study they saw a shifting renal threshold of glucose with an eventual stabilization at 3 months post-op (Bradley, 1973), while in another, later study, they concluded that $T_m$gluc/GFR remained constant at all times post-nephrectomy! (Bradley, 1974)

Attempting to separate function from mass, Silber and Malvin transplanted kidneys into healthy rats. Two months later, these 3-kidney animals had GFR's and RBF's both 50% above 2-kidney controls without any change in size in either of the three kidneys over pre-op values. However, when hypertrophied kidneys from unilaterally nephrectomized rats were transplanted into other unilaterally nephrectomized rats (thus creating rats with two hypertrophied kidneys), six weeks later both GFR and RBF had decreased to that of control values and both kidneys had shrunk to normal size (see section on Alterations in Mass) (Silber and Malvin, 1974).

Finally, micropuncture studies of the glomeruli reveal the mean transcapillary hydraulic pressure difference $\langle \Delta P \rangle$ to be increased from 34 to 40 mm Hg, but the ultrafiltration coefficient (Kf—the product of effective hydraulic permeability and capillary surface area) is unchanged from control (Deen, 1974). In addition, it has been shown that filtrate reabsorption along the entire nephron increased in proportion to the increase

in the single nephron GFR (Emmanouel, 1975; Katz, 1967). Also, Hayslett demonstrated that although filtrate transit time through the proximal tubule is unchanged, the transit time through the distal tubule is prolonged with a shorter Na reabsorption half-time when compared to control (Hayslett, 1968).

Studies in healthy human kidney transplant donors have fortunately provided much more consistent results. Many groups have demonstrated GFR's of 68-75% and RBF's of 62-71% of 2 kidney values one week post-op with a slow leveling off of GFR to 78-92% and RBF to 61-76% of pre-op values at 18 months post-nephrectomy, with no subsequent changes (Krohn, 1966; Donadio, 1967; Ogden, 1967; Flanigan, 1968; Skov, 1974). Pabico showed that during the post-nephrectomy period, T_mPAH, $T_m$gluc, and C_{H_2O} increased in proportion to the increase in GFR while C phosphate, C uric acid and net urinary acid secretion increased disproportionately to the GFR increase (Pabico, 1975). Also, there seems to be an inverse relationship between the subjects' ages and the increases in GFR and RBF (Ogden, 1967), but studies attempting to correlate the increase in GFR with increases in kidney size have been contradictory (Boner, 1972; Skov, 1974).

Thus, although generalizations about <u>early</u> functional changes post-unilateral nephrectomy or ureteral obstruction cannot really be made, it seems safe to state that compensatory increases in GFR and RBF to about 65% of pre-op values are complete by 3-4 weeks post-operatively. Most likely there

is an abrupt increase within the first 48 hours with a gradual, slow increase after that. Preliminary studies also suggest that a greater absolute change in both GFR and RBF is seen following unilateral nephrectomy than is seen post-unilateral ureteral ligation.

Histological Changes

In order to understand the compensatory alterations in mass and function, early workers focused on whether these changes were secondary to an increase in the number of nephrons in the remaining kidney, or were due to some alterations within the structure of the nephron. It has generally been agreed that following nephrectomy in adult animals, there is no change in the number of nephrons in the remaining kidney (Arataki, 1926; Moore, 1929; Kaufman, 1974, 1975), although one lone worker claims that in rats younger than 2 months of age, the number of nephrons did increase (Bonvalet, 1972).

As the evidence overwhelmingly supports the concept that the number of nephrons remains constant, the mass increases should be due to either hypertrophy (increase in the size of the cell) or hyperplasia (increase in the number of cells) or both. Both have been studied extensively with reasonably consistent results. Hypertrophy is generally looked at by making actual measurements on histological sections of the kidneys and then applying some crude approximations for calculations. For example, greatest and least diameters of glomeruli are measured with a micrometer, averaged, and the glomerular volume is calculated as for a sphere. Hyperplasia is considered to be measured by the number of mitoses seen in histological

section, as obviously each mitosis reflects the formation of 2 new cells. However, studies of hyperplasia have been hampered somewhat by the fact that healthy rats have a normal diurnal variation in the number of mitoses in all four regions of the kidney (inner and outer cortex, and inner and outer medulla): the peak number is seen at 2-4 p.m., while the lowest number is seen at 6-8 a.m., with the proximal tubule having the largest absolute number at all times (Blumenfeld, 1938; Williams, 1961).

Although biochemical evidence for early (less than 48 hours post-surgery) hypertrophy has been substantial (see section on Biochemical Alterations), histological evidence has not. In rabbits 2 hours post-nephrectomy, the mean nuclear volume of proximal convoluted tubules in both cortical and juxtamedullary nephrons is decreased from control values, but it is increased at 24 and 48 hours, perhaps suggesting an increase in RNA synthesis necessary for the production of intracellular protein needed for hypertrophy (Fajers, 1957). Parallelling this, within 24 hours of nephrectomy, there is an increase in the diameters of the proximal convoluted tubules. This is secondary to cellular hypertrophy, as the width of the lumens did not change (Rollason, 1949). Unfortunately, more studies on early cellular hypertrophy have not been performed.

Many groups have shown that late hypertrophy (greater than 48 hours post-surgery) does exist and seems to be greatest in the cortex (Jackson, 1927). However, there is some argument as

to whether it is due to increases in the size of the glomeruli (Saphir, 1927), proximal convoluted tubules (Hayslett, 1968; Bradley, 1974) or both (Arataki, 1926). Schubert demonstrated that by one week following unilateral ureteral obstruction in rats, the normal kidney showed maximal swelling of the epithelial cells in both the proximal and distal tubules, accompanied by an increase in the lumen of Bowman's space and an increase in the juxtaglomerular granulation index (Schubert, 1975). Hayslett demonstrated that the proximal tubule increased 35% in length and the distal tubule 17% in length, 2-4 weeks following unilateral nephrectomy (Hayslett, 1968).

Fortunately, studies on <u>hyperplasia</u> have proved to be much more consistent. Virtually all investigators have demonstrated, irrespective of normal diurnal variation, a maximum increase in the number of mitoses (usually 4-6 times control values) at 48 hours following unilateral nephrectomy or obstruction (Rollason, 1949; Saetren, 1956; Ogawa, 1958; Williams, 1961; Goss, 1960; Saetren, 1963; Dicker, 1971) seen mainly in the cortex, but other groups have suggested secondary, smaller peaks of mitoses at 60-75 hours and at 7 days, seen mainly in the outer medulla (Saetren, 1963; Ogawa, 1958). However, these peaks are never confined solely to one region, with the greatest absolute increase seen in the cortex, followed by the outer medulla (Rollason, 1949). Indeed Williams' elegant studies revealed that the proximal convoluted tubules had a

peak number of mitoses at 48 hours, while the ascending limbs had peaks at 40 hours and 72 hours, and the collecting ducts had peaks at 40 hours and 96 hours post-nephrectomy (Williams, 1961).

This hyperplasia which is so predominant within the first few days post-op is not permanent. Many studies have revealed that by one to two weeks after unilateral nephrectomy or obstruction, the number of mitoses has returned to baseline levels (Johnson, 1966; Dicker, 1971; Williams, 1961).

At the subcellular level, at 24 hours post-nephrectomy, there was a 2-fold increase over control in the number of mitochondria, but this decreased to control values at 2 days, suggesting that the number of mitochondria increased to supply the energy needed by the proximal convoluted tubules for early (24 hours) compensatory hypertrophy (see above) (Worthen, 1972). Using electron microscopy, this hypertrophy was shown to involve dilation of the cisternae of rough endoplasmic reticulum, proliferation of the Golgi apparatus and smooth endoplasmic reticulum, an increase in the number of ribosomes, and the appearance of absorption droplets and microtubules polarized along the cell axis (Anderson, 1967).

In summary, then, although cellular hypertrophy is seen histologically 24-48 hours following unilateral nephrectomy or obstruction, cellular hyperplasia is the predominant event noted during this period--with the number of mitoses 4-6 times

control values. These changes occur mostly in the cortex. However, by one to two weeks, the number of mitoses has returned to normal and it is cellular hypertrophy which predominates and is responsible for the mass increase seen at that time. Curiously, there have been no studies comparing histological changes seen in unilaterally obstructed versus unilaterally nephrectomized animals.

Biochemical Alterations

With several exceptions (Ogawa, 1961; Halliburton, 1965; Kurnick, 1968; Miyada, 1960), biochemical studies seemed to correlate well with the histologic observations. Using P^{32} or H^3 thymidine, many groups have noted a maximum uptake into DNA at about 48 hours (Simpson, 1961; Benitez, 1964; Johnson, 1966; Threlfall, 1967; Paulson, 1973) with some workers noting a steady decline for 3 or 4 days (Johnson, 1966; Ogawa, 1961; Threlfall, 1967) with a second smaller peak at 7-9 days (correlating with Ogawa's histological studies) (Simpson, 1961); while others report an overall steady increase in DNA content to 20-25% above control 2-4 weeks post-op (Miyada, 1960; Threlfall, 1964, 1967; Malt, 1968; Paulson, 1973). There seems to be no difference in DNA changes between nephrectomized vs. obstructed animals (Dicker, 1972). Since DNA synthesis is thought to be a marker of cellular hyperplasia, these biochemical studies are quite consistent with histologic observations that compensatory hyperplasia is seen predominately in the first 48 hours post-nephrectomy.

Similarly, studies of RNA and protein synthesis parallel somewhat the hypertrophy seen histologically, but the exact timing is not clear. Johnson has shown that following nephrectomy, both RNA synthesis (incorporation of H^3-cytidine) and protein

synthesis (incorporation of C^{14}-leucine) were sharply increased within the first hour and were both doubled at 3 hours, remaining at that level for 48 hours; thus giving biochemical corroboration to the cellular hypertrophy seen within the first 24 hours--24 hours before hyperplasia occurs (Johnson, 1966). Somewhat in contradiction, Coe claimed that increases in protein synthesis were more biphasic than steady: the first peak occurring 14-24 hours post-op (representing cellular hypertrophy) with the second occurring 24-48 hours post-op (representing both hypertrophy and hyperplasia) (Coe, 1967). When RNA fractions were studied, it was noted that 15 minutes post-nephrectomy, HnRNA (Heterogeneous RNA $>$ 28S) began to decrease, and by 60 minutes was drastically lowered (Willems, 1969). In addition, Malt showed that rRNA synthesis peaked 2 days post-op with a second, smaller peak at 8 days (thus somewhat parallelling mitotic activity), while mRNA synthesis was least at 2 days and greatest at 4 days post-op (the inverse of rRNA production). This sequential change was explained by the notion that new ribosomes had to be synthesized before the transcription of new mRNA could begin (Malt, 1967).

Several groups agree that total RNA continues to rise slowly to 30-40% above control levels at 3-4 weeks following unilateral nephrectomy or ureteral ligation (Malt, 1968; Threlfall, 1967; Potter, 1974; Paulson, 1973), but Kurnick claimed a return to normal levels after only 3 days (Kurnick, 1968).

Studies on kidney RNA/DNA ratios have been more consistent. At 12 hours following unilateral obstruction or nephrectomy, the whole kidney RNA/DNA ratio increased, was 20-40% above control values at 24 hours (Halliburton, 1965; Malt, 1968), peaked at 14 days and then stayed the same or decreased slightly over the following 2 weeks, suggesting hypertrophy accompanying the hyperplasia (Paulson, 1973). Indeed, Johnson claimed that 5 days post-op, compensatory growth was 75% hypertrophy and 25% hyperplasia (Johnson, 1966). Also, it has been shown that changes in tubular RNA/DNA parallel that of whole kidney RNA/DNA (Vancura, 1970) but this appears to occur only in the cortex (Dicker, 1971).

Following unilateral nephrectomy there appears to be no increase in the activity of enzymes in the Krebs cycle of the remaining kidney (Nowinski, 1967). The NA-K-ATPase in the ascending limb of the loop does increase (Kiil, 1972). This correlates well with Hayslett's finding that the reabsorptive half-time of sodium in the distal loop is indeed shortened, suggesting an increased capacity per NA-K pump or an increased number of pumps per unit length (Hayslett, 1968). Also it has been noted that 5 minutes following nephrectomy, the rate of C^{14} choline incorporation into cortex phospholipid increased 37% above control, 68% between 20 minutes and 3 hours, and declined slowly after 2 days (Toback, 1974). This suggested that the transient decrease in fractional sodium reabsorption

seen early following nephrectomy may be secondary to de-differentiation of cell membranes prior to cell growth (Mobasheri, 1975).

In summary, then, the biochemical data both parallels and amplifies the histological data. Apparently, the earliest events following unilateral ureteral nephrectomy or obstruction are those of cellular hypertrophy, with increases in RNA and protein synthesis seen within several hours post-surgery. Later, at about 48 hours, DNA synthesis, mirroring mitoses, overshadows all other events, but then declines over the next several days. Meanwhile the biochemical changes of hypertrophy continue as hyperplasia declines, so that cellular RNA and protein increase to a plateau level 30-40% above control values by about two weeks post-op.

To explain these events, Johnson has proposed that the early cellular changes of hypertrophy (as manifested by an early increase in RNA and protein synthesis) cause the cell to reach a certain "critical mass" at which point the cell automatically divides. The timing involved is such that the maximum number of mitoses is seen 48 hours after the initial event (Johnson, 1966).

II. THEORIES

Over the years, four theories have evolved to explain the above-presented observations. Chronologically, they are

(1). Work-Load (Solute Load) Theory: Loss of functioning renal mass causes a buildup of serum solutes and metabolites whose presence stimulates compensatory alterations in remaining renal tissue.

(2). Blood Flow Theory: Hemodynamic alterations secondary to loss of functioning renal tissue cause an increased blood flow to remaining renal tissue which in turn spurs compensatory growth and function.

(3). Serum Factor Theory: Following removal of renal mass, the remaining tissue secretes a diffusable organ-specific substance into the blood which promotes compensatory alterations.

(4). Removal of Inhibitors: Normal renal tissue secretes substances into the blood which prevent renal growth and function from exceeding a certain level. When renal tissue is injured or removed, the amount of serum inhibitors is lessened, and compensatory increases occur.

The first theory has largely been discounted, and the bulk of recent research has been focused on proving the existence of and isolating renal growth promoting or inhibiting factors.

Work-Load Theory

The work-load theory was both the earliest explanation for compensatory renal function and hypertrophy, and the first to have serious objections raised against it. In the 1920's and '30's, it was the most widely accepted hypothesis, first being suggested by Hinman in 1922 when he noted that unilaterally obstructed kidneys relieved after 3 days undergo greater regeneration when the ureter of the contralateral kidney is partially ligated (Hinman, 1922); and that unilaterally transplanting a ureter into the duodenum leads to marked bilateral hypertrophy (Hinman, 1922; Hartman, 1933). Others have also demonstrated that following unilateral nephrectomy, there is greater weight increase in the remaining kidney if its ureter is anastamosed to bowel than if left alone (Bollman, 1935), while one worker showed marked increases in the size of both kidneys of dogs when human urine, concentrated ten times and/or inorganic salts were injected into the small bowel (Hartman, 1933).

Several groups have shown that following unilateral nephrectomy, both the absolute increase and rate of increase in renal size were greater on high protein diets, suggesting perhaps an effect of increased nitrogenous metabolites (Smith, 1927; MacKay, 1938; Dicker, 1971).

Others, however, have postulated the work-load theory on much more indirect evidence. One group suggested that since

compensatory renal growth secondary to unilateral ureteral ligation or transection was slower than that following unilateral nephrectomy, the concept of an increased sodium load was a viable explanation since the amount presented to the healthy kidney was much more abruptly increased following nephrectomy (Mason, 1965). Johnson and others claimed that the early (within hours) increase in RNA and protein synthesis following unilateral nephrectomy suggested the concept of a "critical mass": the work load presented to the nephron regulates RNA and protein synthesis, and consequently cell size; and that the cell only divides when it reaches a certain critical size or critical ratio of cytoplasmic mass to nuclear mass (Johnson, 1966; Potter, 1969).

In contrast, the evidence against the work-load hypothesis has been greater in volume and more convincing in content. Urea loading in animals via infusion (not diet), following nephrectomy seems not to appreciably increase O_2 consumption, RNA/DNA ratio, or hypertrophy in the remaining kidney over what is seen with nephrectomy alone (Van Slyke, 1934; Halliburton, 1965; Dicker, 1971). Although it has been consistently demonstrated that following unilateral diversion into the peritoneum, or urea loading, the GFR in the normal kidney is 40-60% above that seen following unilateral nephrectomy, in the same experiments there was no increase in renal mass over control levels (Block, 1953; Hayslett, 1972; Diezi, 1973; Weinman,

1971, 1973; Bugge-Asperheim, 1968) and no increase in DNA synthesis as measured by P^{32} uptake (Simpson, 1961). In fact, the early work of Hinman and Hartman has all but been discredited by Block. He showed that in rats three weeks post right ureteroduodenostomy, the contralateral kidney did not enlarge unless there was right uteropelvic dilatation. He attributed the earlier findings to loss of renal tissue secondary to partial ureteral obstruction rather than to a build-up of solutes (Block, 1953).

That the increase in GFR results in rather than is a result of, increased sodium reabsorption was shown by Hayslett. He demonstrated an increase in GFR with pure volume expansion in rats on a salt-free diet. Also by increasing GFR by 50% with methylprednisone injections, he showed an increase in tubular sodium reabsorption without an increase in renal weight (Hayslett, 1972). Indeed Kiil has shown that the increase in sodium absorption is due to increased absorption in the distal nephron only and is not dependent on an increased delivery of solutes from the proximal tubules (Kiil, 1968). Others have also felt that an increase in GFR can be dissociated from a compensatory increase in renal mass and thus an increase in reabsorptive work is not a sufficient stimulus for renal hypertrophy and hyperplasia (Weinman, 1971, 1973; Katz, 1967). In fact, in in unilaterally nephrectomized rats, those fed a diet free of organic salts had the same increase in renal mass as those on a normal diet (Block, 1953). Finally, Malt claims that the

increases in RNA seen following nephrectomy occur too soon (within several hours) to be caused by an increased work load (Malt, 1968).

Blood Flow Theory

Although the concept of increased renal perfusion as a stimulus to compensatory renal hypertrophy and function was a logical offshoot of the work-load hypothesis, this has been the least studied of the four theories. This is probably due to the obvious inherent difficulties in separating blood flow from work-load, and in differentiating blood flow changes as cause vs. effect in compensatory alterations.

As discussed in earlier sections, changes in both whole kidney perfusion and intrarenal blood distribution in the contralateral kidney following unilateral nephrectomy have been reasonably well delineated. Early researchers noted capillary hyperemia (dilatation) following nephrectomy, one group referring to it as the "transient pseudohypertrophy of congestion" (Saphir, 1927; Jackson and Levine, 1929; Carriere, 1973). The many studies showing that contralateral renal blood flow and O_2 consumption following unilateral ablation or obstruction increase dramatically have already been discussed (Van Slyke, 1934; Krohn, 1970; Maluf, 1949; Levy and Blalock, 1938). One of these groups separated work-load from blood flow, demonstrating that renal O_2 consumption in a unilaterally nephrectomized dog was unaltered when blood urea was increased 10-fold over normal via intravenous infusion (Van Slyke, 1934). Another of these

groups demonstrated that following nephrectomy, the increase in renal O_2 consumption parallelled the RBF increase exactly, implying that it was purely secondary to RBF changes, and not because of greater O_2 extraction from the blood (Levy and Blalock, 1938).

Using transducers, Krohn directly measured renal artery blood flow in dogs following contralateral nephrectomy. Within the first 5 minutes, there was a 27% increase in single renal artery blood flow over pre-op values with a gradual increase to 33% at 3 hours post-op; also there was a 25 mm Hg rise in distal aorta pressure post-op. This could be explained by the theory that the ratio of distal aorta to one renal artery blood flow is always 2:1 -- i.e., normally the distal aorta gets 25% of the cardiac output and each renal artery gets 12.5%. Following unilateral nephrectomy, the same amount of blood is redistributed so that the distal aorta gets 33.3% and the remaining renal artery gets 16.7%, thus explaining the above results (Krohn, 1970). Dicker and Shirley felt that this was ample evidence that increased RBF was the initial stimulus for hypertrophy, later changes being caused by other factors (Dicker and Shirley, 1971, 1972).

Schaffenburg showed that in unilaterally nephrectomized rats, renin infusions markedly inhibited compensatory renal hypertrophy (Schaffenburg et. al., 1954). Later workers demonstrated that within three weeks following unilateral ureteral obstruction, the renin content of the obstructed kidney had

doubled over pre-op values and the kininogenase (kallekrein) content had decreased to 20% of pre-op levels (Barton and Schachter, 1974). However, whether this reflects increases of serum levels of kallekrein is not known.

Kaufman demonstrated that rats with 75% of the renal mass removed had a much greater proportional increase in mean nephron RBF (MNRBF) vs. mean nephron GFR (MNGFR) than rats with a simple unilateral nephrectomy, implying decreasing filtration fraction with decreasing renal mass. He also showed that in both groups, there was a disproportionate increase in blood flow to the inner cortex (Kaufman et. el., 1975). However, with micropuncture studies, others demonstrated that following unilateral nephrectomy, single nephron GFR (SNGFR) and single nephron plasma flow (SNGPF) increased in parallel, the mean transcapillary hydraulic pressure difference $\langle \Delta P \rangle$ increased, and the ultrafiltration coefficient (Kf), the product of the effective hydraulic permeability and capillary surface area did not change, thus implying that increases in SNGFR resulted from increases in SNGPF and $\langle \Delta P \rangle$ (Deen, 1974).

Peters showed that during the first 18 hours following unilateral nephrectomy in rats, the total GFR was approximately halved while urine output was similar to pre-op values. That this was secondary to decreased proximal tubule reabsorption was indicated by increased potassium excretion. The author felt that this might be due to renal blood flow alterations (Peters, 1963). Others have also claimed that increased renal

blood flow is responsible for increased delivery of fluid to the distal tubule which is mediated by afferent arteriolar tone (Kiil, 1972). Indeed, some workers feel that this glomerulo-tubular imbalance, from the inability of the proximal tubules to increase Na reabsorption above a certain amount, is the initial cause of renal afferent and efferent arteriolar changes which are mediated via the macula densa-JG apparatus release of renin (Bradley, 1973). This, and proximal tubule volume increases all seem to suggest that preservation of glomerulo-tubular balance might be the prime mediator in compensatory renal function (Bradley, 1974; Hayslett, 1968).

The only work which seems to directly oppose the blood flow theory is that of Potter who hypotonically expanded the ECF in both sham operated and unilaterally nephrectomized rats. He and his colleagues noted that although the GFR in the nephrectomized animals increased as expected over the 24-hour period post-op, that of the shams did not. Unfortunately, there were no non-ECF-expanded, unilaterally nephrectomized controls (Potter, 1974).

Thus, although the blood flow theory is largely based upon indirect evidence, the data against it is sparse. Nevertheless, attention has been diverted from it towards trying to isolate a serum factor and/or prove the existence of an inhibitory substance.

Serum Factor Theory

Since the late 1930's, it has been widely known that endocrine alterations can affect the compensatory response of healthy renal tissue to the loss of functional renal mass. Various groups have noted that hypophysectomy, castration and adrenalectomy seem to depress compensatory renal growth (McQueen-Williams and Thompson, 1940; Goss and Rankin, 1960; Korenchevsky and Ross, 1940; Williams, 1962), while testosterone, thyroxin and growth hormone seem to enhance compensatory hypertrophy (not hyperplasia) (Korenchevsky and Ross, 1940; Korenchevsky and Hall, 1944; White et. al., 1949). As discussed earlier, the amount of protein in the diet has striking effects upon renal growth. Also, other protein moieties have been examined. Renin has been shown to inhibit compensatory renal hypertrophy (but not in hypophysectomized animals) while folic acid seems to enhance hyperplasia (Schaffenburg et. al., 1954; Threlfall et. al., 1967). Kallikrein content in unilaterally obstructed kidneys appears to drop progressively following the onset of obstruction suggesting slow release into the bloodstream of lymphatics, or increased renal matabolism (Barton and Schachter, 1974).

These experiments have probably confused work upon a serum factor theory. The idea of an organ-specific substance

released by non-functional renal tissue (after unilateral ureteral obstruction) or by good renal tissue (after a unilateral nephrectomy) which stimulates compensatory renal growth and function, is not new. In the early 1930's, Breuhaus noted that 14 days post-unilateral nephrectomy, rats that had had intraperitoneal injections of macerated kidney at the time of nephrectomy, had three times the number of renal mitoses as non-injected animals (Breuhaus and McJunkin, 1932). Although later, similar experiments gave somewhat opposite results (see section on Removal of Inhibitors), some investigators continued to look for serum factors.

In in vitro experiments, both rat kidney cultures and renal slices have a much greater number of mitoses and an increased incorporation of labeled thymidine into DNA when incubated with serum obtained from rats 2 days post-unilateral nephrectomy than with serum from control rats (Ogawa, 1958; Lowenstein, 1966; Preuss, 1970). Ogawa showed that serum from rats obtained 15 days post-nephrectomy did not have this effect and that this "mitosis-stimulating factor" was not species-specific (it worked on dog kidney cultures), was heat stable at 56° but destroyed at 100°C and was non-dialyzable (Ogawa, 1958). Paradoxically, later workers showed that the serum effect from nephrectomized rats was destroyed by heating at 56°C or by freezing serum for 72 hours (Lowenstein and Lozner, 1966). Interestingly, Preuss showed that serum from bilaterally nephrectomized rats, unlike that from unilaterally nephrectomized

ones, had no in vitro effect on incorporation of labeled nucleic acids into DNA and RNA. This suggested that a serum factor, if it did exist, originated from renal tissue, and was probably organ-specific because it had no effect on liver slices (Preuss et. al., 1970).

In vivo experiments have really not been any more specific about a serum factor. When mitotic activity in mouse kidney in initially unilaterally nephrectomized animals is compared with that in animals with one kidney damaged with repeated needle insertions, 2 days after the insult there is no difference between the two groups (Argyris and Tremble, 1964). The "organ specificity" of the suggested substance has been reaffirmed by the fact that partial hepatectomies have no effect on the number of mitoses seen following unilateral nephrectomies in rats (Simpson, 1961). One worker has suggested that non-identifiable proteins seen by electron microscopy localized to membrane-bound subcellular particulate matter in the cortex after unilateral nephrectomy, may well be the "serum factor" which resembles ribonuclease and is freely filtered by the glomerulus and reabsorbed by tubules (Royce, 1967).

In vivo experiments with the serum or blood of nephrectomized animals have been a bit more enlightening. When 2-3 ml of blood is removed from rats 30 hours status-post unilateral nephrectomy (without replacement with an equal volume of saline however) the number of mitoses seen at 48 hours post-nephrectomy

is markedly reduced (Goss and Rankin, 1960). However, this may well be due to the effects of volume depletion rather than to removal of a serum factor. When serum from unilaterally nephrectomized animals is injected into normal ones, the uptake of labeled thymidine into renal cells is dramatically increased over control while that into hepatocytes is not, thus reaffirming organ-specificity (Lowenstein and Stern, 1963; Silk et. al., 1967). Working with pairs of mice made parabiotic by anastamoses between the carotid artery of one and the jugular vein of the other, Kurnick studied weight changes in remaining kidneys 10 days following the removal of 1, 2 or 3 kidneys. Removal of one kidney resulted in substantially greater mass increases in the remaining kidney than that seen in the kidneys of the intact partner. Also when one kidney was removed from each mouse of a pair, the growth in remaining kidneys was greater than that seen when both kidneys were removed from one animal. Finally removal of three kidneys resulted in the greatest increase noted. Kurnick concluded that a "serum factor" was present in low concentrations and that it crossed into a parabiotic partner with difficulty (Kurnick and Lindsay, 1968). Similar experiments with parabiotic rats showed no increase in labeled thymidine uptake in remaining kidneys when both kidneys of one animal were removed, suggesting that a "serum factor" was indeed elaborated by renal tissue (Lytton et. al., 1969). Lytton felt that if a serum factor did exist,

it affected compensatory renal hypertrophy and hyperplasia but not initial alterations in GFR and RBF (Lytton, 1972).

The evidence against the serum factor theory has been plentiful and thought-provoking. Several groups have noted striking failures in the ability of serum obtained from unilaterally nephrectomized rats to induce, after injection, increases in the number of mitoses or increases in labeled thymidine uptake in normal rats (Williams, 1962; Reiter and McCreight, 1964; Kurnick and Lindsay, 1967). Finally, some investigators have demonstrated that after unilateral nephrectomy in one rat of a parabiotic pair (made parabiotic either before or after the nephrectomy), the kidneys in the intact rat have shown neither increase in mass nor increase in H^3-thymidine uptake over control (Thomson and Lytton, 1967; Johnson and Vera-Roman, 1968).

Thus, although evidence suggests that an organ-specific serum factor may exist, much of data obtained is by indirect means, and may just as easily be explained by other theories. In many of the above-cited experiments, blood removal and/or tissue removal are important parts of the methods used, and easily invoke theories of blood flow or removal of inhibitors.

Removal of Inhibitors

This theory, an offshoot of the serum factor theory in that it proposes the existence of a serum substance which normally inhibits renal growth, is the most recent of the four theories. Weiss, in the early 1950's, noted less growth of metanephros grown in kidney extract than those grown without extract, while destruction of one member of a pair of metanephros in a pre-functional stage led to a 100% increase in mitotic activity in the remaining one (Weiss, 1952). Following unilateral ureteral ligation in rats, the greater the amount of renal tissue destruction in the obstructed kidney, the greater the contralateral compensatory kidney weight increase, thus suggesting that healthy renal tissue in some way inhibits compensatory changes (Block et. al., 1953). Recent work has demonstrated that removal of 1 1/2 kidneys from rats results in a much greater increase in remaining renal weight and mean nephron GFR (MNGFR) over control than does unilateral nephrectomy alone (Kaufman et. al., 1974).

Saetren showed that following unilateral nephrectomies in rats, if kidney macerates were injected into the peritoneal cavity, the 48 hour post-nephrectomy mitotic wave would be suppressed but subsequent waves would not. Also, the closer to 48 hours the macerate was given, the smaller the amount

needed for suppression, and liver and brain macerates had no effect, implying organ-specificity. If the macerate were heated to 60°C for 10 minutes, there was a loss of the inhibitory effect, but freezing and thawing in a vacuum had no effect. In addition, macerate prepared from kidneys obstructed for 10 days had no inhibitory action (Saetren, 1956, 1963). Others have obtained similar results (Williams, 1962), and recently Dicker showed that macerates prepared from renal cortex suppressed compensatory renal mass increases, increases in protein content, RNA/DNA ratios and O_2 uptake, while macerates from renal medulla did not (neither suppressed compensatory GFR increases) (Dicker, 1972). One group of workers demonstrated that long after unilateral nephrectomy, if rats received transplants of kidneys from unilaterally nephrectomized rats (thus giving the rats two hypertrophied kidneys), 6 weeks later renal weight, GFR, and RBF had all decreased to control values (Silber and Malvin, 1974).

Recently there have been two case reports of unilateral nephrectomies in humans with severely impaired contralateral renal function, following which there was a surprising increase in renal function in the remaining kidneys as measured by creatinine clearance (Schiff et. al., 1974).

There have been, of course, studies questioning the concept of an inhibitor. Goss was able to suppress the 48-hour post-nephrectomy mitotic peak in rats, not only with

peritoneal injections of renal macerate, but also with injections of liver, testis, spleen, blood and egg albumin, thus raising serious doubts about the existence of a tissue-specific inhibitor (Goss, 1963). Indeed, another worker was able to suppress the compensatory 48-hour mitotic peak with intraperitoneal talc injections, suggesting that all the previously-demonstrated inhibition might be secondary only to a non-specific peritonitis (Royce, 1963). Also, one group has shown that in parabiotic rats, bilateral nephrectomy in one member of the pair produced the same increase in renal weight and RNA/DNA ratio as did a unilateral nephrectomy (Van-Vroonhaven, 1972).

At this time, the removal of inhibitors theory appears to be the most likely of the four theories--not because it has the strongest data support of the four but because it is the least refutable. Indeed, the contradictory evidence seen in attempts to prove each of the theories gives rise to the suggestion that perhaps some combination might explain compensatory renal changes. For example, could a serum factor function as a vasodilator and thus induce renal growth secondary to increased blood flow? Or could a usually present substance check overly large renal blood flow, until there is decreased production of this substance by loss of functioning renal tissue? Or might not both a serum factor and an inhibitor exist—the latter blocking the effects of the former until renal tissue loss

supervenes? As can be seen, the potential for further conjecture is greater than substantial evidence in support of just one theory.

EXPERIMENTS

The purpose of these studies was to examine compensatory alterations seen in renal mass, GFR and RBF following unilateral ureteral obstruction or unilateral nephrectomy in Sprague-Dawley rats, and to note differences, if any, between the two groups.

Veeder, in this lab, had previously compared these two groups 21 days post-surgery, and noted that both renal mass and GFR were significantly greater in unilaterally nephrectomized rats than in unilaterally obstructed animals (Veeder, 1975). The new studies are an attempt to determine whether these are immediate events (that is whether immediately post-op there is a difference between obstructed and nephrectomized animals), or whether there is a gradual separation between these two groups over time. Thus in the following experiments, the rats were studied 2, 12 and 30 days after initial surgery. Also, by comparing the effects of nephrectomy versus those seen with retained, nonfunctioning renal mass (i.e., an obstructed kidney), some new data on the <u>cause</u> of compensatory renal changes is sought.

Materials and Methods

Male Sprague-Dawley rats (Charles River Breeding Company) were used in all of the experiments. The rats, which weighed between 150 and 300 grams, were paired by weight so that there was no more than a 10 gram weight difference between each member of a pair.

Under ether anesthesia (Mallinckrodt), one rat of the pair underwent a right nephrectomy while the other had the right ureter obstructed. The nephrectomy was performed via a right flank incision, with freeing up of the kidney and ligation of the pedicle with 2-0 silk before cutting the kidney away. The kidney was then stripped of its renal capsule, patted dry and weighed. The flank muscles were sutured with 2-0 silk with a superficial layer of skin clips. The ureteral obstruction was performed via a midline supra-pubic, vertical abdominal incision. The right ureter was exposed and ligated with 2-0 silk. The abdomen was then closed with 2-0 silk and a superficial layer of skin clips.

Upon awakening, the rats were placed in individual cages and were pair-fed between 10 and 20 grams daily of standard laboratory chow (Purina) which had a 23% protein content and 17.2 mEq/100 grams of sodium. They were allowed water ad lib.

The pairs were studied either 2 days, 12 days or 30 days following the nephrectomies and obstructions. The rats were weighed and then anesthetized with an intraperitoneal injection of Inactin (Promonta, Hamburg) of 80-100 mg/kg. Following induction of anesthesia the rats were shaved and placed on heating boards where their temperatures were maintained between 36° and 37.5°C as measured by rectal probe. Via neck incision, polyethylene tracheostomy tubes (Intramedic PE 205) were inserted, along with left external jugular vein lines (Intramedic PE 50). Additionally, the obstructed rats had left carotid artery lines inserted (Intramedic PE 50).

Via midline supra-pubic incisions, the bladders were exposed, freed up, and polyethylene collecting tubes (Intramedic PE 50) were inserted via small incisions at the avascular apex of the bladder to catch the urine.

Fluid losses were replaced with 0.9% saline at .22cc/min (Harvard pump) via the jugular vein line until 2% of the initial body weight was given. Following this a bolus of 10μ Curies of inulin-methoxy H^3 (New England Nuclear Corporation) was given through the jugular line and then an infusion of 0.2 μ Ci/min was started (Harvard pump) in a total volume of 1.2 ml.

The rats were equilibrated with the inulin infusion for 45 minutes and then, while continuing the infusion, three 10-minute clearances were run. Urine was collected via the polyethylene catheter and measured. Halfway through each collecting

period, arterial blood was obtained from incisions in the rats' tails and was spun down to determine hematocrits. After the last 10 minute collecting period, left renal vein blood was obtained with a 25 gauge needle. This too was spun down and the hematocrit was measured. The intact (left) kidney was removed, stripped of its renal capsule, and weighed, and the animals were then sacrificed.

Aliquots of urine (1μl) were placed in 10cc of aqueous counting scintillant (Amersham/Searle) while 20μl aliquots of the spun plasma were placed in separate scintillant solutions. Each scintillation vial was counted for 10 minutes by a Tri-Carb liquid scintillation spectrometer (Packard), along with 2 blank controls, for H^3. As the amount of urine (1μl) and plasma (20μl) in each vial was known, the concentrations [U]in and [P]in could be calculated. Also as urine had been collected in 10 minute runs, urine flow V (μl/min) was easily calculated.

Inulin clearance, Cin, whch is approximately equal to the glomerular filtration rate, GFR, was calculated by the formula:

$$Cin = \frac{[U]in \cdot V}{[P]in}$$

The clearances were then divided by the rats' weights (in hundreds of grams) to obtain the clearance in μl/min/100 grams. This was done for each of the three 10 minute collection

periods and then the average clearance or average GFR/100 grams body weight of each rat was determined.

Inulin extraction, (E), the percentage of inulin removed from the plasma by the kidney, was calculated from the formula

$$Ein = \frac{[A]in - [V]in}{[A]in}$$

where [A]in is arterial inulin concentration and [V]in is renal vein inulin concentration. Since Cin = Ein · RPF, where RPF is the renal plasma flow, the RPF was derived from

$$RPF = \frac{Cin}{Ein}$$

Since RPF = (% of blood that is plasma) · RBF, where RBF is renal blood flow, RBF was easily calculated from the formula

$$RBF = \frac{RPF}{(1 - Hct)}$$

as the hematocrits had been measured. The RBF's were then divided by the rats' weights (in hundreds of grams) to obtain blood flow in μl/min/100 gm. This was done for each 10 minute collection period and the three were averaged to get an average RBF for each rat. This method of calculating renal blood flow obviously measures blood flow only to the functioning left kidney.

The percent change in left kidney weight was calculated from the formula

$$\% \text{ change} = \frac{\text{final kidney weight} - \text{initial kidney weight}}{\text{initial kidney weight}} \times 100$$

The initial weight of the left kidney in both nephrectomized and obstructed rats was estimated from the weight of the right kidney removed from the nephrectomized animal. This would appear to be feasible because it has been shown that right and left kidneys in the same animals have almost identical weights, and that rats with the same body weight (as these animals paired by weight did) have kidneys of similar weight (Kaufman, 1974). Wet kidney weights were used, as dry weight is always 24% of wet weight, making either parameter as useful as the other for study (Malt and Lemaitre, 1968). Also by dividing the kidney weights by the rats' final body weight, the percent increases in left kidney weight per 100 grams body weight were calculated.

In addition to the above experiments, blood flow studies were performed on the obstructed rats before they were sacrificed. A bolus of 120,000 microspheres labeled with Sr^{85} ($15 \pm 2\mu$, 3M Corporation) was injected via the left carotid artery. After several minutes, both kidneys were removed, stripped of their renal capsules and weighed. They were then placed in separate scintillation vials and each vial, along with two blanks, was counted for one minute by a gamma counter (Beckman). As the microspheres are trapped in the kidneys during a single passage in proportion to the blood flow to them

(Arruda and Boonjaren, 1974), the blood flow to the obstructed right kidney could be estimated by the calculation:

$$\text{right RBF (per 100 grams B.W.)} = \frac{\text{right kidney counts}}{\text{left kidney counts}} \times \text{left RBF (100gm B.W.)}$$

These studies were undertaken to see what changes, if any, occurred with time in blood flow to the unilaterally obstructed kidney, and how they related to changes in blood flow to the functional kidney during the same time period.

All of the results obtained from nephrectomized rats were compared with those from their obstructed partners using both a Student's t-test (utilizing differences of the means) and paired t analysis (utilizing means of the differences). With both tests, results were considered statistically significant if $p < 0.05$.

Results

Rats were studied 2 days, 12 days and 30 days following the initial surgery and the changes in the nephrectomized animals were compared with those in the obstructed rats at each of the time intervals.

The body weight changes and left kidney weight changes in paired rats studied 2 days following right unilateral ureteral obstruction or nephrectomy are shown in Table I. In the thirteen pairs and two single rats studied, the average initial body weights were identical at 248 $\pm$ 5 gm, but two days following surgery the obstructed rats had an average weight of 232 $\pm$ 4 gm and the nephrectomized rats had an average weight of 238 $\pm$ 5 gm. These values were not significantly different with Student's t-test ($p > 0.05$). The initial estimated left kidney weights were identical between members of a pair because, as discussed earlier, they were defined as equal to the weight of the right kidney removed at nephrectomy (Kaufman, 1974). The obstructed rats had an average final left kidney weight of 1.097 $\pm$ .035 gm while the nephrectomized rats had an average of 1.161 $\pm$ .039 gm. Although a trend appeared, these values were not statistically significant using both Student's t-test and paired t analysis. The average percent increase in left kidney weight in obstructed animals was 10.6 $\pm$ 2.2% and 11.5 $\pm$ 1.8% in nephrec-

% INCREASE IN LEFT KIDNEY WEIGHT

OBST	NEPH
11.02	9.06
	7.94
19.34	5.72
13.12	24.85
9.33	14.40
.61	13.96
	7.49
1.08	10.90
10.16	11.08
6.75	21.45
	6.35
22.31	5.26
12.46	
10.62	11.54
2.19	1.80
.3251 (p = n.s.)	
.6598 (ρ = n.s.)	

Fold Out

tomized rats. These values also were not statistically significant. Thus at 2 days post-surgery a slightly greater, but not statistically significant increase in renal mass appears to occur in nephrectomized vs. obstructed animals.

Eleven pairs and four single rats were studied 12 days after surgery (Table II). The average initial weight of the obstructed rats was 197 ± 7 gm and that of the nephrectomized ones was 196 ± 6 gm. The average final body weights were not significantly different: obstructed equal to 239 ± 8 gm, nephrectomized was 238 ± 7 gm. The average estimated initial left kidney weights were, of course, identical. The obstructed rats had an average final left kidney weight of 1.210 ± .045 gm, and the nephrectomized animals had one of 1.241 ± .043 gm (not statistically significant). The average percent increase in left kidney weight in obstructed rats was 42.3 ± 5.6% while that of the nephrectomized ones was 43.8 ± 4.1%. These also were not statistically significant by both Student's test and paired t analysis.

Ten pairs and seven single rats were studied 30 days following right unilateral ureteral obstruction or nephrectomy as shown in Table III. The average initial body weights were not statistically significant using Student's t-test: obstructed 163 ± 5 gm, nephrectomized 160 ± 5 gm. Neither were the average final body weights: obstructed 275 ± 7 gm, nephrectomized 274 ± 5 gm. The average initial estimated left kidney weights

TABLE II 12 DAY RATS

RAT #	INITIAL BODY WEIGHT (GM)		FINAL BODY WEIGHT (GM)		LEFT INITIAL KIDNEY WEIGHT (GM)		LEFT FINAL KIDNEY WEIGHT (GM)		% INCREASE IN LEFT KIDNEY WEIGHT	
	OBST	NEPH	OBST	NEPH	OBST (est)	NEPH	OBST	NEPH	OBST	NEPH
1	170	169	191	193	.730	.730	.930	.973	27.40	33.29
2		186		221		.751		1.147		52.73
3	194	187	225	179	.749	.749	1.118	.906	49.27	20.96
4	251	248	265	245	1.011	1.011	1.325	1.218	31.06	20.47
5	172	164	222	213	.758	.758	1.307	1.248	72.43	64.64
6		192		255		.886		1.450		63.66
7	196	193	269	233	.825	.825	1.467	1.292	77.82	56.61
8		211		258		1.022		1.483		45.11
9	222	213	240	263	.858	.858	1.213	1.236	41.38	44.06
10		228		285		1.095		1.463		33.61
11	210	205	250	245	1.019	1.019	1.313	1.381	28.85	35.53
12	216	213	269	272	1.036	1.036	1.258	1.302	21.43	25.68
13	190		218		.822		1.150		39.90	
14	192	186	245	244	.860	.860	1.054	1.264	22.56	46.98
15	160	160	210	223	.779	.779	1.030	1.122	32.22	44.03
16	193	180	264	245	.825	.825	1.349	1.136	63.52	69.81
mean =	197.2	195.9	239.0	238.3	.8560	.8698	1.2095	1.2414	42.32	43.81
S.E. =	7.2	6.2	7.5	7.4	.0312	.0348	.0450	.0434	5.59	4.05
t-test =	.1361 (p = n.s.)		.0697 (p = n.s.)		.2960 (p = n.s.)		.5095 (p = n.s.)		.2160 (p = n.s.)	
paired t=					1.0000 (p = n.s.)		.6257 (p = n.s.)		.1168 (p = n.s.)	

TABLE III 30 DAY RATS

RAT #	INITIAL BODY WEIGHT (GM)		FINAL BODY WEIGHT (GM)		LEFT INITIAL KIDNEY WEIGHT (GM)		LEFT FINAL KIDNEY WEIGHT (GM)		% INCREASE IN LEFT KIDNEY WEIGHT	
	OBST	NEPH	OBST	NEPH	OBST (est)	NEPH	OBST	NEPH	OBST	NEPH
1	127	127	228	230	.712	.712	1.011	1.220	41.93	71.35
2	152	154	304	265	.708	.708	1.222	1.276	72.60	80.23
3	151	146	267	267	.708	.708	.974	1.373	37.57	93.93
4	152	151	258	260	.656	.656	1.314	1.194	100.30	82.01
5		168		292		.801		1.468		83.27
6		161		279		.803		1.856		131.13
7		163		292		.768		1.349		75.65
8	162	160	254	289	.930	.930	1.562		67.96	
9	169	161	263	282	.746	.746	1.340	1.307	79.62	75.20
10	190	183	299	283	.803	.803	1.752	1.303	118.18	62.27
11	159	152	258	264	.740	.740	1.206	1.309	62.97	76.89
12	160	154	290	291	.712	.712	1.318	1.304	93.96	83.15
13	180	203	265	274	.805	.805	1.524		127.80	
14		158		274		.761		1.394		83.18
15	152		265		.732		1.295		76.91	
16	174		314		.730		1.532		109.86	
17	194		303		.825		1.394		68.97	
mean =	163.2	160.1	274.5	274.4	.7439	.7609	1.3418	1.3622	81.43	83.19
S.E. =	5.0	4.7	7.0	4.5	.0200	.0178	.0604	.0496	7.63	4.89
t-test =	.4600 (p = n.s.)		.0039 (p = n.s.)		.6331 (p = n.s.)		.2673 (p = n.s.)		.1936 (p = n.s.)	
paired t =					1.0000 (p = n.s.)		.2123 (p = n.s.)		.1889 (p = n.s.)	

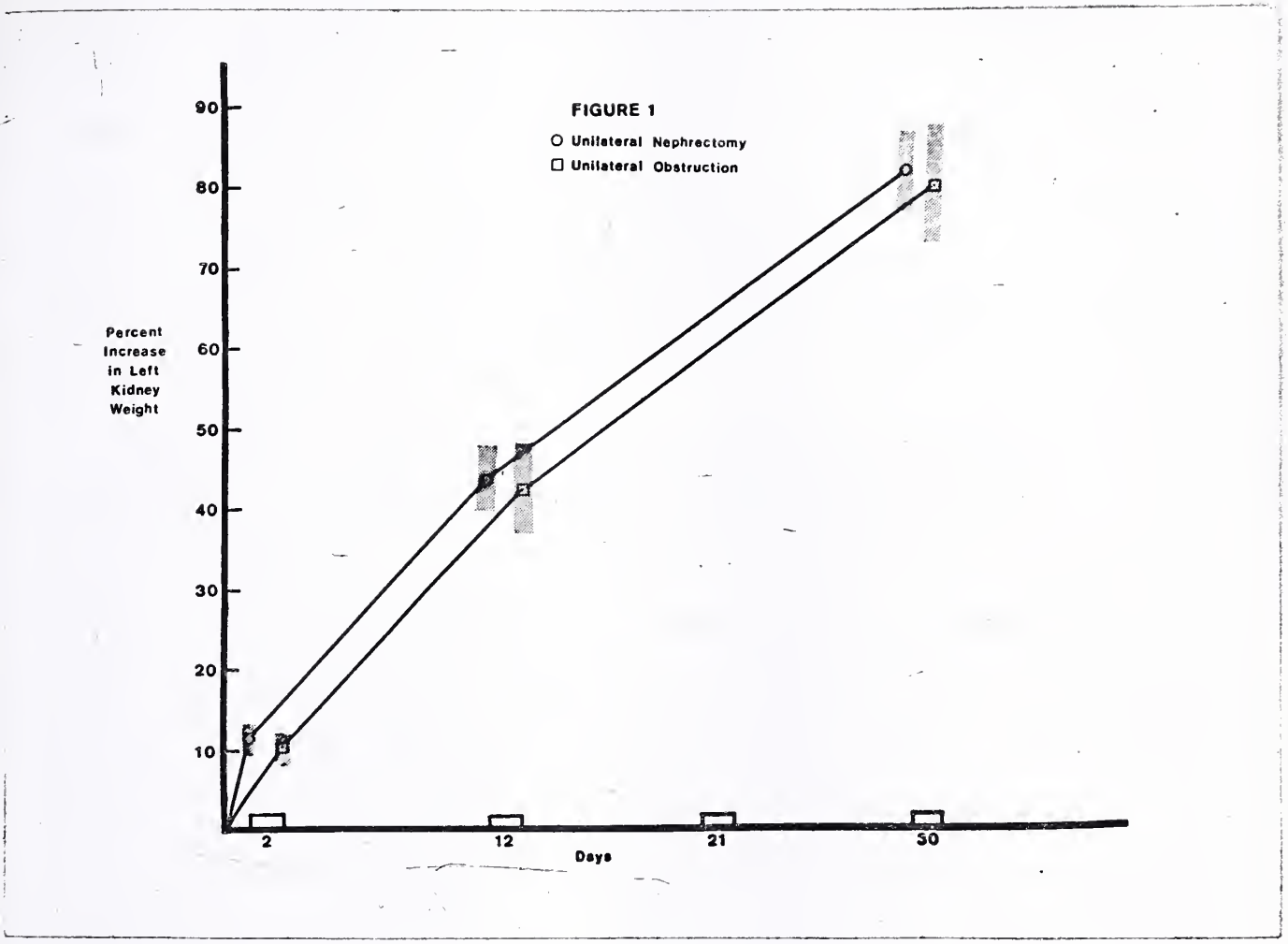

Figure 1. Percent increase in left kidney weight vs. time (days) post-surgery.

were by definition not significantly different. The obstructed animals had an average final left kidney weight of 1.342 ± .060 gm while the nephrectomized ones had an average final kidney weight of 1.362 ± .050 gm. These were not statistically significant by Student's t-analysis. Finally, the average percent increase in left kidney weights in obstructed rats was 81.4 ± 7.6% while that of nephrectomized animals was 83.2 ± 4.9% (not statistically significant).

The percent increases in left kidney weight plotted against time for both unilaterally obstructed and nephrectomized rats is shown in Figure 1. At time zero, there is obviously 0% increase in left kidney weight. As is shown, there is no appreciable difference between obstructed and nephrectomized rats at each of the three time points. Both groups had a steady increase in left kidney weight, the sharpest increases between 0 and 2 days, and 2 and 12 days; the slope flattening somewhat between 12 and 30 days.

The clearance and blood flow data of paired rats studied 2 days following right unilateral obstruction or nephrectomy are shown in Table IV. In the ten pairs and five single rats studied, the unilaterally obstructed rats had an average clearance of 670 ± 25 μl/min/100 gm while the unilaterally nephrectomized rats had an average clearance of 726 ± 36 μl/min/100 gm. Both Student's t-test and paired t-analysis gave p values that were not significant ($p > 0.05$). The obstructed rats had an average

TABLE IV 2 DAY RATS

RAT #	$\bar{V}'$ (μl/min/100 gm)		GFR (μl/min/100 gm)		EXTRACTION		RBF (μl/min/100 gm)		Right RBF (μl/min/100 gm) (microspheres)
	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST
1	1.52		749		.16		7545		
2	1.47	5.05	672	869	.40	.35	3220	4428	
3	2.44		609		.37		3096		2577
4		6.09		850		.15		11651	
5	2.25	1.74	857	719	.21	.28	9718	5151	
6	3.28	3.10	736	993	.30	.19	5267	10868	2252
7	8.05	3.05	583	609		.21		5507	
8		3.12		633		.33		3650	
9	1.73	3.31	692	562	.31	.10	4333	10630	
10	3.05	2.41	595	747	.33	.32	3616	4439	1725
11	1.54	1.34	587	884	.28	.33	4233	5097	
12	2.65	2.54	748	648	.25	.39	5658	3066	1706
13		1.87		730				5422	
14	1.88	3.61	628	577	.38	.31	3153	3161	1617
15	5.63	1.36	584	612	.33	.40	3290	2813	1157
mean =	2.96	2.97	670	725.6	.302	.280	4829.9	5837.2	1839.0
S.E. =	.57	.38	25.4	37.8	.022	.028	639.6	862.7	205.0
t-test =	.0159 (p = n.s.)		1.2209 (p = n.s.)		.6157 (p = n.s.)		.9379 (p = n.s.)		
paired t =	.4895 (p = n.s.)		1.0478 (p = n.s.)		.3690 (p = n.s.)		.8954 (p = n.s.)		

RBF of 4830 ± 640 μl/min/100 gm, while the nephrectomized animals had one of 5837 ± 863 μl/min/100 gm. Again both tests gave p values that were not significant.

With the 12-day rats (Table V), seven pairs and eight single animals were studied. The average GFR in the obstructed rats was 697 ± 11 μl/min/100 gm while the nephrectomized animals averaged 736 ± 50 μl/min/100 gm. Statistical analysis with both t-tests proved these values not to be significant. The average RBF in the obstructed animals was 4441 ± 222 μl/min/100 gm while that of the nephrectomized ones was 4656 ± 626 μl/min/100 gm. These too were not statistically significant.

With the seven pairs and ten single rats studied 30 days following right unilateral obstructions or nephrectomies, some statistically significant data was obtained (Table VI). The obstructed animals had an average GFR of 537 ± 37 μl/min/100 gm and the nephrectomized animals had one of 680 ± 23 μl/min/100 gm. The Student's t-test produced $0.001 < p < 0.005$ for these two numbers, although the paired t-test produced a p-value that was not statistically significant. The average RBF of the obstructed rats was 4119 ± 464 μl/min/100 gm while that of the nephrectomized animals was 3789 ± 322 μl/min/100 gm (not significant).

The inulin clearance plotted against time for both unilaterally obstructed and nephrectomized rats is shown in Figure 2. Earlier work in this laboratory revealed 2-kidney control animals to

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TABLE V 12 DAY RATS

RAT #	$\bar{V}'$ (μl/min/100 gm)		GFR (μl/min/100 gm)		EXTRACTION		RBF (μl/min/100 gm)		Right RBF (μ/min/100 gm) (microspheres)
	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST
1	4.80	2.89	631	1097	.25	.24	5704	9672	
2		8.02		669					
3	8.30		685		.28		4958		1379
4	1.70	6.61	685	798	.31	.31	3366	5011	
5		1.77		750		.35		3708	
6	1.75	1.70	756	786	.27	.41	4417	3449	1861
7		2.43		503		.32		3101	
8	6.21		712		.32		4129		1145
9		10.56		863		.31		4987	
10	2.35	2.05	670	745	.27	.27	5130	4702	1006
11	3.85		693		.30		4531		1713
12	24.53		665		.30	-	4377		
13	3.21	1.73	723	501	.36	.19	3537	5548	
14	2.78	1.99	731	684	.29	.41	4264	3091	
15	1.93	2.27	718	704		.39		3295	
mean =	5.58	3.82	697.2	736.4	.295	.320	4441.3	4656.4	1420.8
S.E. =	1.99	.93	10.6	49.6	.010	.023	222.4	626.0	162.6
t-test =	.8007 (p = n.s.)		.7722 (p = n.s.)		.9964 (p = n.s.)		.3237 (p = n.s.)		
paired t =	.1202 (p = n.s.)		.7209 (p = n.s.)		.2934 (p = n.s.)		1.0134 (p = n.s.)		

TABLE VI 30 DAY RATS

RAT #	$\bar{V}'$ (μl/min/100 gm)		GFR (μl/min/100 gm)		EXTRACTION		RBF (μl/min/100 gm)		Right RBF (μl/min/100 gm) (microspheres)
	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST	NEPH	OBST
1	5.65	1.95	460	776	.22	.31	6747	5309	
2	4.87	4.14	633	636	.17	.33	7619	3685	
3	1.13	2.03	441	570	.32	.39	2654	2863	
4	2.16	1.99	662	743	.32	.38	3149	3932	
5		2.30		729		.44		2962	
6		1.84		666					
7		2.33		767		.36		4183	
8	7.80		654		.29		4132		389
9	2.14	5.18	664	713	.38	.31	3803	4583	
10	10.63	2.96	722	559	.27	.37	4588	2785	493
11	2.66	1.38	311	748	.15	.26	4257	5710	245
12	2.11		471		.26		3676		472
13		3.37		561		.41		2528	
14		1.78		696		.42		3142	
15	1.80		422		.22		3759		956
16	2.22		517		.34		2950		
17	2.20		485		.42		2099		149
mean =	3.78	2.60	536.8	680.3	.280	.362	4119.4	3789.3	450.7
S.E. =	.84	.32	36.5	23.4	.024	.016	463.5	321.6	114.7
t-test =	1.3083 (p = n.s.)		3.3110 (0.001 < p < 0.005)		2.8483 (0.005 < p < 0.01)		.5852 (p = n.s.)		
paired t =	1.0734 (p = n.s.)		1.6124 (p = n.s.)		2.7534 (p.025 < p < 0.05)		.7806 (p - n.s.)		

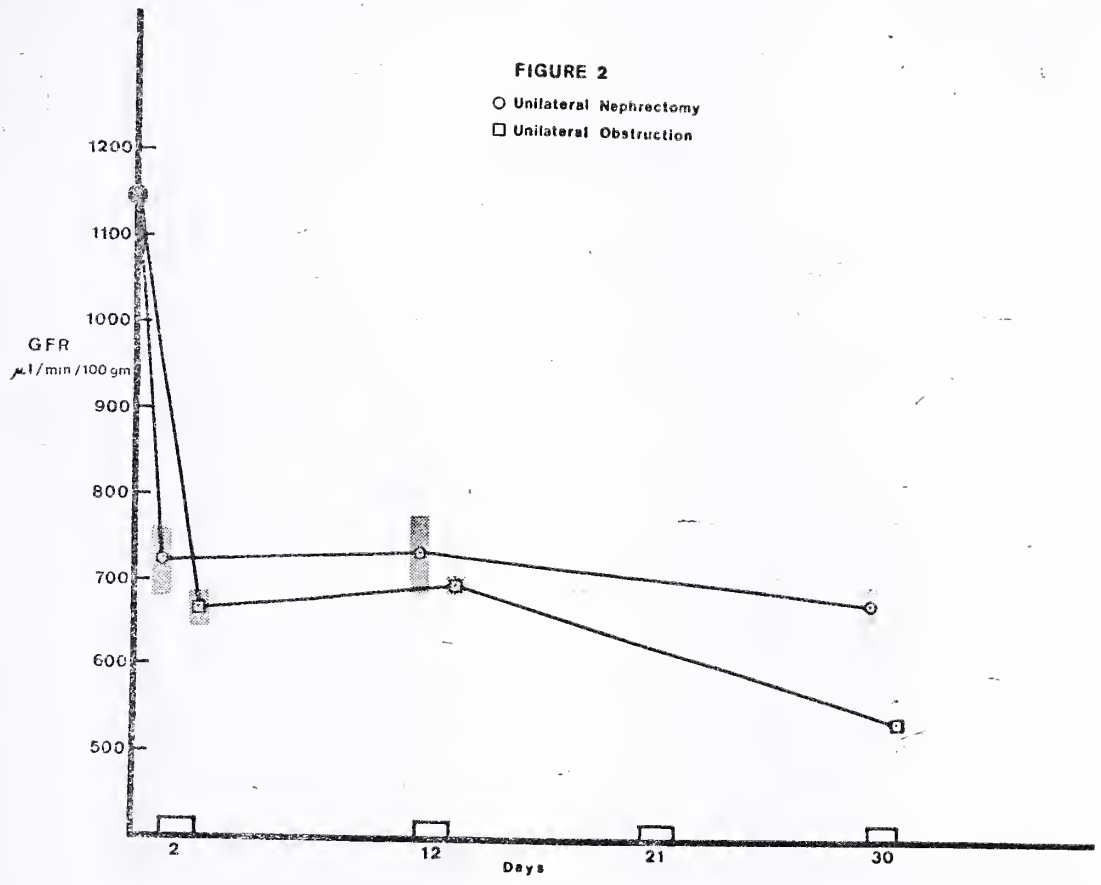

Figure 2. Glomerular filtration rate (μl/min/100 gm body weight) vs. time (days) post-surgery. Control GFR (from a healthy two-kidney rat) at time zero was 1144 + 48 μl/min/100 gm body weight (Kaufman et al., 1976).

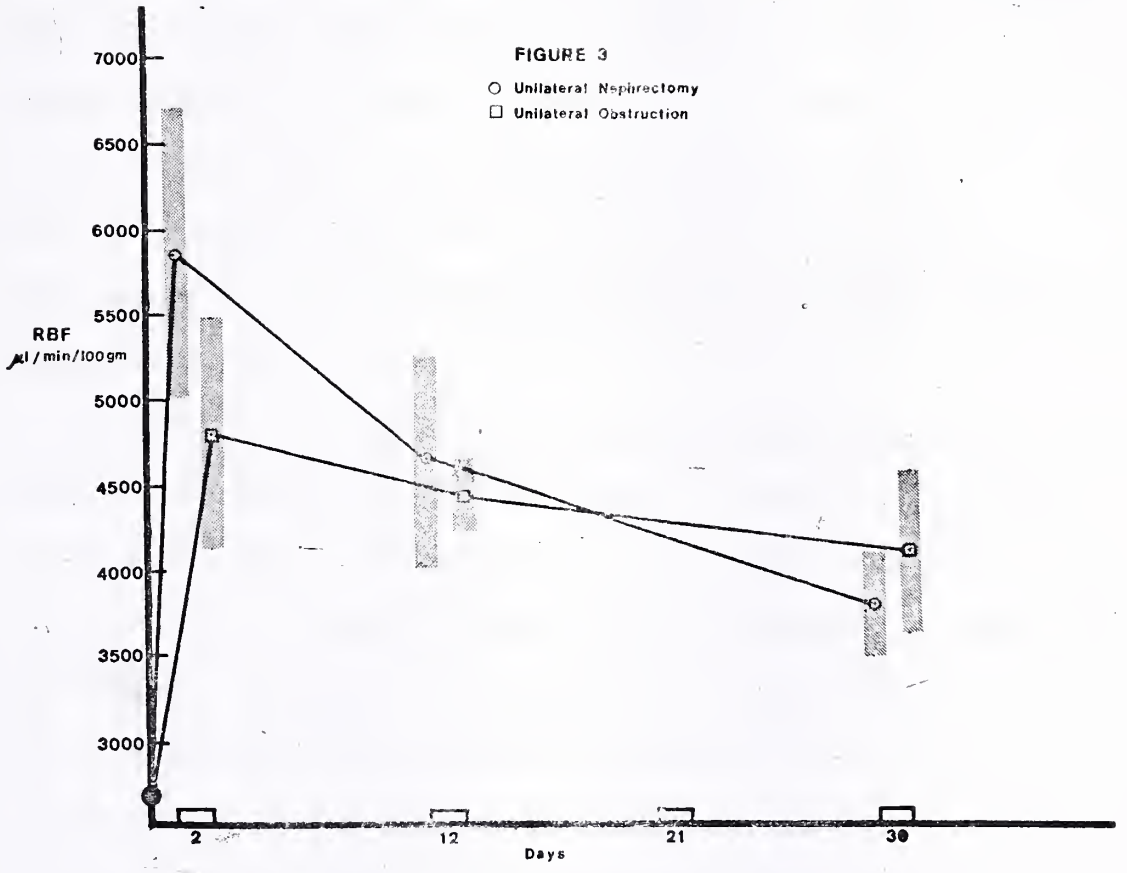

Figure 3. Left renal blood flow (μl/min/100 gm body weight) vs. time (days) post-surgery. Control RBF to the left kidney at time zero was taken as 50% of the RBF of a healthy two-kidney rat — 2645 $\pm$ 98 μl/min/100 gm (Kaufman et al., 1976).

have a GFR of 1144 ± 48 μl/min/100 gm body weight (Kaufman et. al., 1976), and this value was used for time zero. As is shown, there is a dramatic drop in GFR during the first 2 days following surgery in both sets of animals, but no further decrease between 2 and 12 days. The nephrectomized animals maintain this GFR between 12 and 30 days, but that of the obstructed rats gradually drops off so that by 30 days it is statistically significantly less.

The renal blood flow to the left kidney plotted against time for both groups of animals is shown in Figure 3. The flow to the left kidney at time zero (i.e., the control value) was taken as 50% of 2-kidney rat renal blood flow previously determined in this laboratory. This value for a single kidney is 2645 ± 98 μl/min/100 gm body weight (Kaufman et. al., 1976). As can be seen, 2 days following the initial surgery, there is a steep increase in RBF to the intact left kidney in both obstructed and nephrectomized rats, with the obstructed rats leveling off at this level while the nephrectomized animals appear to have a slightly decreasing RBF between 2 and 30 days.

As discussed earlier, additional labeled microsphere experiments were performed on obstructed rats to determine blood flow to the obstructed right kidney. The data are shown in Tables IV, V, and VI. The six rats studied 2 days following obstruction had an average right renal blood flow of 1839 ± 205 μl/min/100 gm body weight. The five rats studied 12 days

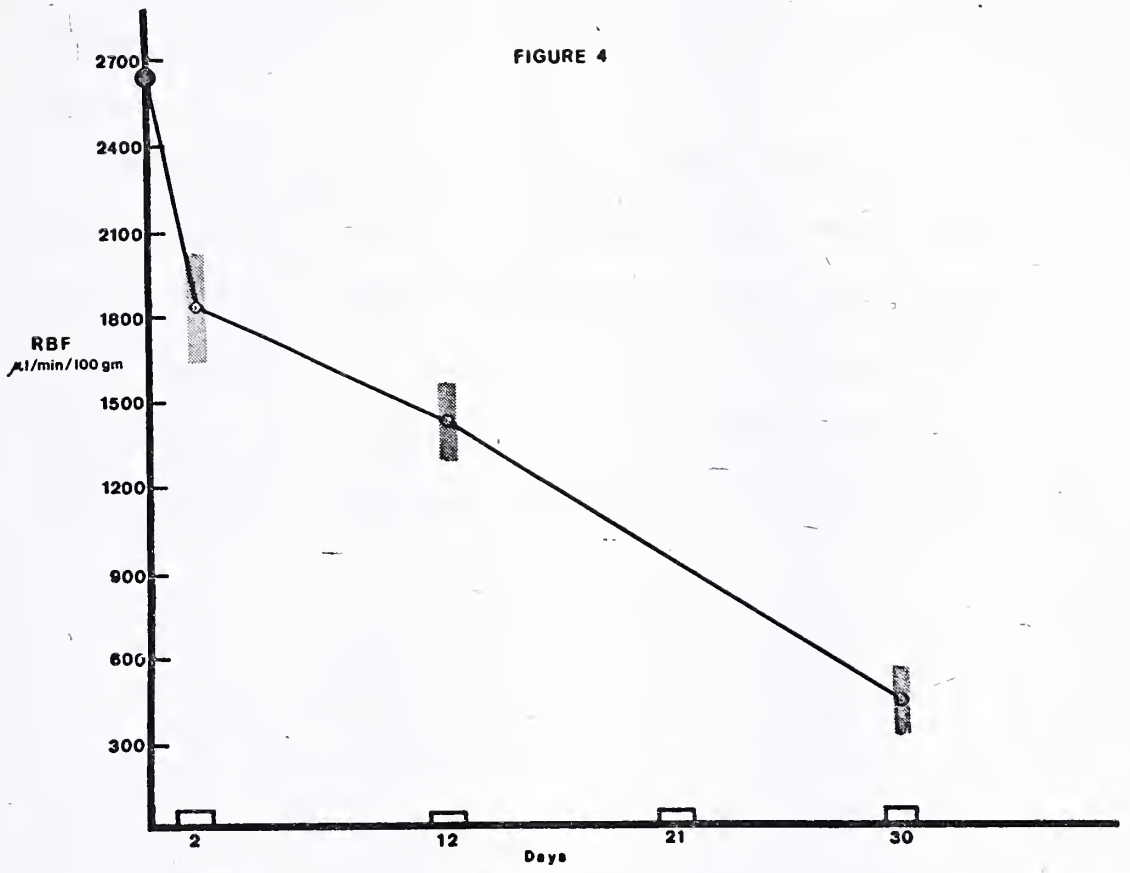

Figure 4. Right renal blood flow (µl/min/100 gm body weight) in rats with right ureteral obstruction vs. time (days) post-obstruction. Control RBF to the right kidney was taken as 50% of the RBF of a healthy two-kidney rat -- 2645 ± 98 µl/min/100 gm (Kaufman et al., 1976).

post-obstruction had an average of 1421 ± 163 μl/min/100 gm body weight, and the six studied 30 days following surgery had an average right renal blood flow of 451 ± 115 μl/min/100 gm body weight.

The blood flow to the obstructed right kidney plotted against time is shown in Figure 4. As explained above, the control value of RBF to a single healthy kidney at time zero is 2645 ± 98 μl/min/100 gm. As is shown, there is a dramatic drop in blood flow during the first two days following obstruction, with a steady, more gradual decrease between 2 and 30 days.

Discussion

Compensatory increase in renal mass secondary to unilateral ureteral ligation compared with that seen following unilateral nephrectomy is shown in Figure 1. The striking point is that between 0 and 30 days post-op, there are no real differences in the percent increases in left kidney weight between the two groups of animals, and thus the slopes of the lines between the time points are virtually identical. Both obstructed and nephrectomized rats have the sharpest increases in left kidney weight during the first 12 days with steady, although more gradual increases between 12 and 30 days. Indeed, as demonstrated by Student's analysis (Tables I, II, III), there was essentially no difference in absolute weights of the intact left kidney between obstructed and nephrectomized rats at any of the three time periods. Yet, the degree of compensatory growth in nephrectomized animals is consistently greater than that in obstructed rats.

This data is at variance with that of Mason and Ewald. When comparing unilaterally obstructed versus nephrectomized rats, they showed that contralateral kidney weights in obstructed animals increased at a much slower rate than in the nephrectomized animals, but that the obstructed rats' kidney weights caught up to those nephrectomized at 24 days post-op. However,

it must be pointed out that not much care was given to separating rats according to weights, nor was rigorous pairing or control of food intake done (Mason and Ewald, 1965). This is important as the amount of dietary protein may effect final renal weight (Smith and Moise, 1927; MacKay, 1938; Dicker, 1971).

Although Dicker and Shirley obtained data virtually identical to that seen at 2 days in Figure 1 (i.e., about a 10% increase in left kidney weight in both groups of animals), their nephrectomized rats continued the sharp increase while their obstructed animals slowed somewhat so that by 7 and 14 days post-op there was significant splay between the growth curves of the two groups -- the nephrectomized rats having greater growth increases than the obstructed ones. Unfortunately the study did not continue past 14 days following initial surgery, nor were the animals paired or paired-fed (Dicker and Shirley, 1972).

Veeder's work in this lab, using virtually identical techniques to those discussed in Materials and Methods, showed that at 21 days post-op, the contralateral kidney had increased 65% in the unilaterally obstructed rats, while there was an 80% increase in the unilaterally nephrectomized animals (Veeder, 1975). This raises the question as to whether or not the growth curve for obstructed animals plateaus out between 12 and 21 days and then rises sharply, so that by 30 days the obstructed animals have the same increase in contralateral renal weight as the nephrectomized ones.

Changes in glomerular filtration rate secondary to unilateral ureteral obstruction compared with that following nephrectomy are shown in Figure 2. As can be seen, there is a sharp drop in clearance in both groups of animals during the first two days post-op, with GFR's remaining at the 2-day values until 12 days following initial surgery. However, the GFR of the nephrectomized rats remains at this level during the ensuing time while that of the obstructed rats drops off gradually so that at 30 days post-op, there is significant splay between the GFR in these groups of animals. This is entirely consistent with Veeder's work from this lab, which at 21 days post-op found the GFR in unilaterally obstructed rats to be significantly less than that of unilaterally nephrectomized animals (Veeder, 1975). From Figure 2 it is obvious that in nephrectomized animals, the GFR value at 2 days is the value that persists for the remaining time period (i.e., 30 days) while the obstructed animal still has the potential to alter its GFR (i.e., it drops betewen 12 and 30 days).

Other workers have obtained somewhat conflicting results. Katz and Epstein, using rats, showed a substantial (25%) increase in total GFR between 3 and 7 days post-unilateral nephrectomy, with an additional 10% increase between 7 and 21 days (Katz and Epstein, 1967). On the other hand, Rous and Wakim, working with dogs, demonstrated the GFR following unilateral nephrectomy declined steadily over the ensuing 28 days (a drop of 15%). They then noted a gradual increase in GFR of 20% between 28 days

and 8 weeks post-op (Rous and Wakim, 1967). However, in partial agreement with Figure 2 are the data from Lyrdal and Olin, who studied rabbits following unilateral ureteral obstruction. They noted that the GFR did not change between 1 and 21 days post-op (i.e., there was no drop-off between 12 and 21 days as seen in Figure 2) (Lyrdal and Olin, 1975).

There have been only three studies comparing GFR in obstructed versus nephrectomized animals over time intervals. The earliest, by Peters, using rats, resulted in identical GFR vs. time graphs in both groups of animals over a 28 day time course. That is, in unilaterally nephrectomized and obstructed rats, there was no change in clearance between 2 and 14 days post-op, but both groups had steady 30% increases between 14 and 21 days, where they remained. However, the two groups of animals were in no way paired and were allowed unlimited food and water. Despite this both groups had equal absolute values for GFR (Peters, 1963). Dicker and Shirley's work also resulted in identical GFR vs. time curves for unilaterally obstructed and nephrectomized rats. Both groups had an initial peak in GFR at 48 hours (equal to control) with a gradual decrease of 30-40% over the next 5-6 days. These animals also were not paired and did not have food or water intake controlled (Dicker and Shirley, 1971, 1972). More recently, Olesen and Madsen performed experiments on unilaterally obstructed and nephrectomized dogs. The animals were initially studied 2 weeks post-op. Between 2 and 4 weeks, GFR

in both groups decreased steadily and then rose gradually over a 2 year period. The rate of increase in GFR after 4 weeks was considerably slower in the obstructed group than in the nephrectomized group. Once again, however, the animals were not paired nor was strict attention paid to control of feeding (Olesen and Madsen, 1975a, b). Thus the data presented in Tables IV, V, and VI, and graphed in Figure 2, seem to be the only studies performed comparing obstructed and nephrectomized paired animals over a time interval.

Indeed, comparing Figure 1 with Figure 2 reveals an obvious separation between mass and function. Although Figure 1 reveals that the left kidney weight in both obstructed and nephrectomized animals rose steadily over the 30 day period (Tables I, II, and III reveal virtual equality in absolute renal weight values between the two groups), Figure 2 shows the obstructed rats' GFR to drop off considerably between 12 and 30 days. This implies that increasing renal mass does not mean increasing GFR -- function is not dependent on mass (Weinman, 1971). Although this does not contradict Katz and Epstein's observation that in unilaterally nephrectomized rats, increases in renal weight preceded increases in GFR, it does question any extrapolation of that statement (Katz and Epstein, 1967).

That the drop in clearance of the functioning left kidney in unilaterally obstructed rats compared to that in unilaterally nephrectomized rats is not due to left renal blood flow differences

is shown in Figure 3. Essentially both groups had a sharp increase in left RBF over the first 2 days post-op, and then a gradual decrease between 2 and 30 days to a value that was still substantially greater than pre-op values. This seems to contradict Van Slyke's work with unilaterally nephrectomized dogs which showed a rising RBF one week post-op, reaching a maximum one month following surgery (Van slyke, 1934). Levy and Blalock, also working with nephrectomized dogs, demonstrated that RBF increased most rapidly during the first month post-op, and then increased slowly, reaching a maximum by about 4 months (Levy and Blalock, 1938). On the other hand, Vaughan, studying unilaterally obstructed dogs, demonstrated a slow rise in RBF over the 6 days post-op (Vaughan et. al., 1970).

However, more in agreement with Figure 3 is the work of Rous and Wakim with unilaterally nephrectomized dogs. They noted that 24 hours post-nephrectomy, the RBF had increased 31% over control, but this value declined steadily over the next 28 days by 23%. They then noted a slow rise in RBF between 4 and 8 weeks post-op (Rous and Wakim, 1967).

There have been only two studies actually comparing contralateral RBF in obstructed vs. nephrectomized animals. Olesen and Madsen, experimenting with dogs, noted that with nephrectomy, the RBF increased slowly by about 40% over control over the 2 weeks post-op and stayed there subsequently, while with unilateral obstruction, the contralateral RBF rose very slowly, reaching the

nephrectomized values only after 52 weeks! (Olesen and Madsen, 1975a,b) On the other hand, at 21 days post-op, Veeder obtained RBF's in unilaterally obstructed and nephrectomized rats that were not significantly different (Veeder, 1975).

With regards to Figures 2 and 3, since both groups had roughly equal GFR and RBF at 2 and 12 days post-op, by definition (see Materials and Methods) they had equal extractions at these two time points -- 28-32% (Tables IV, V). In light of the equal RBF's at 30 days, the differences in inulin clearance may be explained by either a decrease in the extraction in obstructed rats or an increase in the extraction in nephrectomized animals. In actuality what has happened (Table VI) is that the obstructed animals' extraction has stayed the same (28%) while the extraction of the nephrectomized animals has increased to 36%--a difference which is statistically significant using both Student's t-test and paired t-analysis. This may be interpreted such that despite a dropping left renal blood flow in unilaterally nephrectomized rats, between 12 and 30 days, the intra-renal blood flow is redistributed so that there is more flow to the glomeruli. This does not appear to occur in unilaterally obstructed animals.

This increase in nephrectomized animals' extraction seems to be borne out by Deen's experiments. Using micropuncture techniques to study single nephron GFR (SNGFR) and single nephron plasma flow (SNGPF) in rats 16-29 days following unilateral nephrectomy, SNGFR was noted to increase parallel to SNGPF as

the mean transcapillary hydraulic pressure difference in the glomeruli increased from 34 to 40 mm Hg (Deen et. al., 1974).

Opposed to this is Kaufman's experiments on rats 4 weeks following unilateral nephrectomies versus 75% renal mass ablation. He demonstrated a much greater proportional increase in mean nephron RBF vs. mean nephron GFR in rats 75% ablated compared to rats with a unilateral nephrectomy (50% ablation), implying a decreasing filtration fraction with decreased renal mass. He felt that this was secondary to a disproportionate increase in blood flow to the inner cortex as shown by microsphere studies (Kaufman et. al., 1975).

Finally, although the results shown in Figure 4 are generally outside the scope of this thesis, they will be mentioned briefly. Following unilateral ureteral obstruction, the RBF to the obstructed kidney drops sharply over the first 2 days, with a steady decrease between 2 and 30 days to a value which is about 25% of control. Although many studies have demonstrated a steady drop in RBF to obstructed kidneys (Herdman and Jaco, 1950; Idborhn and Muren, 1956; Harris et. al., 1974; Lyrdal and Olin, 1975; Moody et. al., 1975), the results seen in Figure 4 come closest to those of Vaughan which in obstructed dogs, had an ipsilateral RBF of 29% of control at 6 days (Vaughan et. al., 1970).

With regards to explanations for all of the above discussed changes in renal weight, GFR, and RBF, certain conclusions may be drawn.

These experiments do not give direct support to the blood flow theory. This conclusion is based on the fact that contralateral renal weight rises in both obstructed and nephrectomized animals as the RBF declines, and even more important is that the GFR in nephrectomized rats is maintained at a fixed level while the GFR drops in obstructed animals. As the extraction in these kidneys is increased, there are obviously intrarenal mechanisms for redistributing blood flow within the kidney to maintain GFR. Thus, the relationship between GFR and RBF becomes somewhat complex and dependent on concomitant changes in cortical blood flow distribution.

The results of these experiments may be explained, however, by either the removal-of-inhibitors theory or by the serum factor theory, with the former presenting a somewhat stronger case. Although in both groups of animals, over the thirty day time period, the changes in left renal weight and RBF were virtually equal, between 12 and 30 days post-op there was a significant increase in the GFR in the nephrectomized group over that of the obstructed group. This may suggest that the physical removal of the right kidney was responsible for the decrease in the serum level of a diffusable compound which inhibits compensatory increases in renal function, while the presence of the non-functional renal tissue following ureteral ligation allows for higher serum levels of this substance.

On the other hand, it may well be argued that if a serum factor is released by healthy renal tissue, the presence of non-functioning renal mass might somehow prevent or inhibit the release and/or effect of this factor. Thus rats with unilateral ureteral obstruction could have smaller compensatory changes than unilaterally nephrectomized ones. Obviously, this serum factor explanation is much more convoluted than that using the concept of removing inhibitors which seems to fit the results a bit more smoothly. However, the data does not lend itself to full support of either theory, and either could be vigorously argued.

Summary

The various alterations in mass, function, histology and biochemistry seen in compensatory adaptation were reviewed along with each of the four theories proposed to explain these changes: the work-load theory, the blood flow theory, the serum factor theory and removal of inhibitors.

New experimental data was presented in which unilaterally obstructed Sprague-Dawley rats were compared with unilaterally nephrectomized ones 2, 12, and 30 days post-operatively. The changes seen in renal weight and RBF at each of these three time points was the same in the two groups. However, at 30 days, unilaterally nephrectomized rats had significantly greater GFR's than obstructed animals. The significance of this was discussed as well as its relationship to previous data. Also several explanations of this phenomenon were entertained.

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